Attraction of Phyllopertha horticola (Coleoptera, Scarabaeidae) males to fragrance components of Ophrys flowers (Orchidaceae, section Fuciflorae)

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The North-European scarabaeid beetle *Phyllopertha horticola* has been observed to visit and exhibit pseudocopulatory behaviour on the nectarless flowers of *Ophrys* species (section *Fuciflorae*). To evaluate the role of chemical signals in the beetles' attraction response to the flowers and to determine the active compounds involved, males from a swarming population on Öland (Sweden) were tested for their attraction to different odour samples: hexane extracts of *Ophrys* flower labella, hexane extracts of male and female beetles, and different blends of compounds identified in *Ophrys* flower fragrances. Male beetles were most strongly attracted to a blend of short-chained aliphatic alcohols present in extracts of *O. holocericea* labella.

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Introduction

The scarabaeid beetle *Phyllopertha horticola* (L.) has repeatedly been observed to visit flowers of *Ophrys* L., section *Fuciflorae* Rchb.f. (Kullenberg 1973, Warncke & Kullenberg 1984, Borg-Karlson 1987). The often white or cerise sepals of *Ophrys* flowers are similar in coloration to the beetles' food plants *Cistus* (Cistaceae) and *Crataegus* (Rosaceae). It has been suggested that these colours may act as visual cues encouraging both male and female beetles to visit and forage on *Ophrys* flowers and thereby incidentally pollinate them (Ferlan in lit. to Kullenberg, Kullenberg 1961). However, recent observations (Forster, Paulus, in lit. to Kullenberg 1976, 1982, 1983) revealed that *P. horticola* males behave as if sexually stimulated when visiting *O. holocericea* (Burm. fil.) Greuter [= *O. fuciflora* (Cranz) Moenck] flowers. Other scarabaeid beetles, such as *Tropinota squalida* Scop. and *Oxythyrea funesta* Poda, also visit and pollinate various *Ophrys* species in the section *Fuciflorae* (Kullenberg 1961).

It has not been established whether the beetles are attracted/excited primarily by odour, or whether visual and tactile cues are also necessary to elicit the complete sequenced behavioural responses leading to pollination by pseudocopulation.

The purpose of this study was to experimentally examine, using behavioural tests, the role of olfactory stimuli in the pollination relationship between the beetle *Phyllopertha horticola* and the species *Ophrys holocericea, O. scolopax* Cav. and *O. tenthredinifera* Willd.

Materials and methods

The study took place on Öland, one of the large islands in the Baltic Sea close to the mainland of southern Sweden. Odour attraction tests were performed from end May to mid June 1983–85 on a field population of beetles inhabiting the border of a grass-lined gravel road surrounded by hazelbushes (*Corylus avellana* L.).

To test the beetles attraction to different

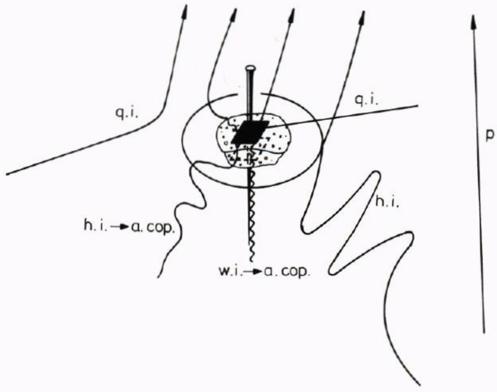

Fig. 1. The odour-guided responses of *Phyllopertha horticola* male beetles are divided into four behaviour types based on increasing degrees of attraction/excitation: (1) *p* = uninfluenced passage, (2) *q.i.* = quick inspection, (3) *h.i.* = hovering inspection and *w.i.* = walking inspection, (4) *h.i.–a.cop.* = hovering inspection followed by "copulation attempt" or *w.i.–a.cop.* = walking inspection followed by "attempted copulation". The insects are regarded as strongly attracted in the behaviour types 3 and 4.

Det doftinducerade beteendet som trädgårdsborrehanar utför vid attrappen är uppdelat i fyra olika typer med stigande attraktivitetsgrad: (1) *p* = passage, (2) *q.i.* = snabb inspektion, (3) *h.i.* = sidsvängande eller *w.i.* = krypande inspektion, (4) *h.i.–a.cop.* eller *w.i.–a.cop.* = sidsvängande eller krypande inspektion som slutar med försök till kopulation på attrappen. Insekterna är starkt attraherade om de reagerar med beteendetyp 3 eller 4.

odours, eight different samples were presented to beetles within the confines of the swarm. Extracts were studied of *Ophrys scolopax* and *O. tenthredinifera* labella, and of female and male *Phyllopertha horticola* bodies. Fresh *Ophrys* labella were extracted in redistilled hexane (5 labella/ml hexane, Merck) for three days at 4°C and 1–2 days old *P. horticola* beetles were extracted in redistilled pentane (2 whole bodies/ml pentane, Merck) for 24 hours at 20°C.

The following three authentic blends of compounds identified in scents of *Ophrys* flowers were studied (volumes in μl; each blend diluted in 1.9 ml hexane): *Alcohol blend*: 1-hexanol 2, *cis*-3-hexen-1-ol 2, 1-octanol 10, 1-hexadecanol 10; *Acid blend*: pentanoic acid 2, hexanoic acid 4, heptanoic acid 10, octanoic acid 2, nonanoic acid 20; *Ophrys tenthredinifera extract blend*: 1-hexanol 2, *cis*-3-hexen-1-ol 2, 1-octanol 10, 1-hexadecanol 10, 6-methyl-5-hepten-2-one 6, pentanoic acid 2, hexanoic acid 4, heptanoic acid 10, octanoic acid 2, nonanoic acid 20, citral 4, geraniol 1, citronellol 4, geranylacetone 4.

The compositions of the floral blends were based on GC-MS (gas-chromatography mass spectrometry) analyses made earlier (Kullenberg et al. 1984, Borg-Karlson 1987).

The test samples were applied to dummies—pieces of black velvet (5×10 mm) placed on insect pins and covered with green tulle. Above the tulle was attached an odourless piece of velvet to increase the visual and tactile stimuli of the dummy. Samples were tested one by one in random order, for 5 minutes each, and in 4–10 replicates. Responses of beetles, within 0.2 m from the dummy, were noted. Results were evaluated statistically using the chi-square test for 2×2 contingency tables. For each test sample, the sum of behaviour types 3 and 4 [hovering (h.i.), walking-inspection (w.i.), and alighting responses on the dummy (h.i.–a.cop., w.i.–a.cop.)], i.e. the strongly attracted beetles (Fig. 1), was divided by the total number of responses (N). Portions of the behavioural sequences were filmed using a Paillard Bolex 16 mm film camera.

Voucher beetle specimens are deposited at the Ecological Research Station of Uppsala University on Öland. Taxonomy and nomenclature of *Ophrys* is based on Nelson (1962), see also Borg-Karlson (1987) and references therein.

Results

In the mornings, male beetles started to walk when ambient temperature reached 13°C (at 1.5 m above ground) and to swarm at 14–15°C. Swarming usually lasted for about three hours. Female beetles were never seen swarming, but were frequently observed feeding on leaves and grasses in the vicinity.

Only males were attracted to the dummies. The behavioural responses of male beetles, both flying and walking, to the dummies were classified into 4 types as follows, listed in order of increasing attraction/excitation (Fig. 1).

1. *p* = uninfluenced passage: by-passing without decreasing flight speed or changing flight direction.
2. *q.i.* = quick inspection: flight direction changed towards dummy, sometimes resulting in touch.
3. *h.i.* = hovering inspection: meandering flight towards the odour source, hovering in front of it, sometimes flying around it, always facing the dummy. *w.i.* = walking inspection: walking on ground or in vegetation towards dummy but without touch.

Tab. 1. Responses of *Phyllopertha horticola* males towards each of the eight samples tested: f(3+4) = the proportion of attracted beetles with behaviour type 3 and 4 (e.g., h.i., w.i., h.i.–a.cop., w.i.–a.cop.) in relation to all beetles (within 0.2 m of the test dummy) for a given sample; f(4) = the proportion of attracted beetles with behaviour type 4; N = the total number of beetles observed for a given test sample; n = the number of replicates for each sample.

Trädgårdsborrens beteendesvar gentemot 8 testämnen: f(3+4) = proportionen attraherade hanar med beteendetyp 3 eller 4, jämfört med alla hanar inom 0.2 m från test-attrappen; f(4) = proportionen attraherade hanar med beteende typ 4; N = totala antalet observerade skalbaggar; n = antal testtillfällen för varje ämne.

Sample	f(3+4)	f(4)	N	n
Alcohol blend	0.36*	0.19*	64	10
Ophrys scolopax labella extract	0.25*	0.10	40	8
Phyllopertha horticola male body extract	0.14	0.09	58	10
P. horticola female body extract	0.13**	0.06	70	8
O. tenthredinifera extract blend	0.12	0.10	50	10
O. tenthredinifera labella extract	0.08	0.00	25	4
Acid blend	0.06	0.02	54	9
Hexane blank	0.05	0.02	56	10

* significantly different from the blank (X^2, $p<0.025$)

** significantly different from the Alcohol blend (X^2, $p<0.05$).

4. *h.i.–a.cop.* = hovering inspection followed by descent and walking on dummy. The beetle performs movements that appear to represent a copulation attempt. *w.i.–a.cop.* = walking inspection followed by contact with the dummy and seemingly attempted copulation.

Extracts of *O. scolopax* labella, which are dominated by the alcohols 1-hexanol and 3-hexen-1-ol (Borg-Karlson 1987), proved to be highly attractive to *P. horticola* males (Tab. 1). The strongest attraction, however, was elicited by the alcohol blend. This blend differed significantly in attractivity from the *P. horticola* female extract (X^2, $p<0.05$), the acid blend and the hexane blank (X^2, $p<0.01$), which released the two weakest responses. Attraction to the extract blend of *O. tenthredinifera,* which contained constituents of both the alcohol and acid blends, was much lower than that to *O. scolopax* extracts, but similar to those observed for body extracts of male and of female *P. horticola*. When a highly excited male beetle responds to the alcohol blend the abdomen is stretched out, the copulation apparatus fully elongated, and the beetle is eagerly grasping the piece of velvet. This behaviour must be interpreted as attempted copulation, and has not been observed when the beetle is feeding on its food plants (Kullenberg pers. comm.). The very few approaches displayed by beetles towards the control hexane dummy suggest that the visual stimulus of the velvet might be negligible compared to the olfactory stimuli of the tested odour samples in eliciting copulation.

Discussion

Results of the present odour-attraction tests indicate that *P. horticola* males are attracted to volatile compounds present in flower fragrances of the studied *Ophrys* species. White and cerise colour cues were excluded in the experiments and the beetles seemed to be only slightly, if at all, affected by the visual stimulus of the dummy on which odours were applied. Only males were attracted to the test-sample dummies and, when highly excited, they performed movements that may be interpreted as "pseudocopulation". Aliphatic alcohols seem to be of greatest importance in releasing this behaviour.

The body extract of female beetles was only weakly attractive to males which is in contrast to the results from a recent study of *P. diversa* Waterhouse where the female body extract was very attractive to conspecific males. This may, however, be influenced in part by the age of the females. The dilution of the *P. diversa* female extract was found to be of minor importance (Kawasaki & Tamaki 1985).

The aliphatic alcohols tested are not restricted in occurrence to the three *Ophrys* species examined here, but are also found in large amounts in the fragrance emitted from *O. lutea* Cav. and *O. fusca* Link (Borg-Karlson et al. 1985, Borg-Karlson & Tengö 1986). However, to my knowledge there are no observations of *P. horticola* visiting these *Ophrys* species, which may be

explained by differences in the blooming times and habitats of different plant species and in swarming times of different beetle populations. *P. horticola* belongs to a family that are known to respond to a variety of compounds (Nilsson A. pers. comm.). 1-Hexanol and *cis*-3-hexen-1-ol, both present in the alcohol blend, are common in plants and probably function as food attractants to many insects. However, an odour induced sexual attraction to the aliphatic alcohol blend and to the *Ophrys* labella extracts is strongly suggested by three observations: (1) beetles oriented towards the odouriferous dummy in the absence of white or cerise colours (visual stimuli), (2) only males were attracted to the dummies, (3) the behaviour of the descending males on the odouriferous dummy resembled the initiation of a copulation. This behaviour was not initiated by the hexane dummy (tactile stimuli not enough).

It is interesting to note that the pollination of *Ophrys* orchids in certain habitats are performed by insects other than bees. The importance of beetles as pollen vectors and their flower preference patterns in the genus *Ophrys*, are not known. Their flower visits are most likely of low specificity, leading to interspecific pollen transfer and may explain some of the hybrids found. The regular pollen vectors in the *Ophrys* section *Fuciflorae* are known to be males of the solitary bees *Eucera* and *Tetralonia* (Kullenberg et al. 1984a, b).

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Sammanfattning

Ophrys orkidéer pollineras i huvudsak av solitära bi-, gräv- och dolkstekelhanar (Hymenoptera: Andrenidae, Anthophoridae, Megachilidae, Sphecidae och Scoliidae). Under senare år har även många observationer av besökande och pollinerande skalbaggar av familjen Scarabaeidae rapporterats inom *Ophrys* sektionen *Fuciflorae*. Dessa orkidéers vita eller rödaktiga kalkblad har antagits ha stor betydelse för insekternas orientering till blommorna. Skalbaggens beteende på den finludna blomläppen har beskrivits både som näringssök och försök till kopulation.

För att utvärdera kemiska signalers betydelse för skalbaggarnas orientering mot *Ophrys*-blommorna, gjordes beteende-experiment i fält på Öland. Åtta olika extrakt och substansblandningar testades på trädgårdsborrar (*Phyllopertha horticola*) vilka har observerats som pollinerare på *O. holocericea* (= *O. fuciflora*). Insekternas reaktioner inför doftattrappen graderades i fyra beteendekategorier med stigande attraktivitet vilka sedan användes för att bedöma attraktionsgraden av olika testämnen.

Endast hanar attraherades, starkast till en

blandning bestående av *cis*-3–hexen-1-ol, 1-hexanol, 1-octanol och 1-hexadecanol (ämnen som också finns i blomdoften hos *O. holocericea* och *O. scolopax*), men inte till attrappen med enbart lösningsmedel. Beteendet på attrappen med alkoholblandningen kan tolkas som sexuellt upphetsat då kopulationsapparaten var helt utdragen hos vissa högexciterade hanar. Trädgårdsborrens hanar attraheras utan visuella stimuli av flyktiga doftämnen som utsöndras från *Ophrys*-labellen. Dessa attraktiva ämnen finns hos flera *Ophrys*-taxa, vilket möjligen kan orsaka korspollination och delvis förklara uppkomsten av vissa hybrider.

Recensioner

Gärdenfors, U., Hall, R., Hansson, C. & Wilander, P. 1988. *Svenska småkryp. En bestämningsbok till ryggradslösa djur utom insekter.* Studentlitteratur. Lund. 147 s. ISBN 91-44-27301-0. Pris 125 SKr.

Bra bestämningslitteratur på svenska är något vi entomologer inte är bortskämda med. Svenska småkryp är därmed ett mycket välkommet bidrag för oss som är intresserade av den lägre faunan. Till skillnad från de flesta populära översikter har författarna valt att ge bestämningsnycklar till samtliga familjer, och inte bara presentera ett axplock vanliga arter. På detta sätt luras man inte till att driva sin bestämning längre än till vad boken egentligen är avsedd för. För den vetgirige kan detta i sig vara frustrerande, men då får man leta vidare i något av de många verk som refereras till efter varje nyckel.

Som titeln anger behandlas ej insekterna, men alla övriga svenska evertebrater. Som regel har man valt att behandla flertalet artrika grupper med småväxta former mer översiktligt. Detta gäller bl a kvalstren och de lägre kräftdjuren, till vilka inga familjenycklar ges. En introduktionsnyckel leder vidare till separata nycklar för olika phyla, vilka sedan även de ofta indelas i undergrupper med separata nycklar. För varje familj ges uppgifter om artantal och utbredning i Sverige, storlek, levnadsmiljöer och vanligen några morfologiska kännetecken utöver de som finns i nycklarna.

Nycklarna är rikligt illustrerade med teckningar av djur i helfigur eller av detaljer. Det är genomgående hög kvalité på teckningarna, som är till mycket stor hjälp.

Boken kommer säkert att användas flitigt inom undervisning. I förordet nämns även att den bygger på kompendier som använts på kurser vid Lunds universitet. Säkert kommer den även att glädja många allmänt naturintresserade, både som hjälp att identifiera djur och som informationskälla.

En sannolik källa till problem för användare är att i nycklarna blandas morfologiska karaktärer med sådana som avser levnadssätt och -miljö. Det senare kan ofta vara svårt att avgöra när man väl har djuret i sin hand. Extra svårt blir det när djur från övergångsmiljöer som t ex brackvatten skall undersökas. Hur väljer man då mellan marina eller limniska former? Det kan även vara svårt att tillämpa de könsbundna karaktärer som används, t ex för vissa av de högre kräftdjuren, när det inte anges hur man skiljer könen åt.

Trots dessa mindre anmärkningar fungerar säkert nycklarna bra, och det är bara att lyckönska författarna till ett fint resultat. Boken är mig veterligt den enda i sitt slag på den svenska marknaden och därmed mycket välbehövlig.

Anders Nilsson

Baccetti, B. (Ed.) 1987. *Evolutionary Biology of Orthopteroid Insects.* Ellis Horwood Series in Entomology and Acridology. Chichester. ISBN 0-7458-0208-7. Pris 1190 DKr.

Initiativet till denna samlingsvolym togs redan 1960 under den Internationella Entomologkongressen i Wien. Det visade sig att det fanns ett stort behov av att samla forskare som arbetade med systematik och fylogeni hos Orthoptera *sensu lato* till ett eget möte. Ditintills hade de flesta konferenser i princip uteslutande behandlat tillämpad acridologi, dvs gräshoppsbekämpning med besläktad problematik. Att det sedan skulle ta mer än 25 år innan mötet blev av hade nog inte initiativtagaren Baccio Baccetti tänkt sig.